Intra-ovarian trichomes in *Bequaertiodendron magalismontanum*: location, origin, structure and possible function in the reproductive process

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Bifurcate, unicellular epidermal trichomes are characteristic of the Sapotaceae, but have only been reported on external surfaces of aerial plant organs. In *Bequaertiodendron magalismontanum* (Sond.) Heine & J.H. Hemsl. such trichomes were found inside the pistils of mature female and morphologically hermaphroditic flowers and inside young fruits. It is suggested that the intra-ovarian trichomes serve as bridges between stylar and ovarian transmitting tissue to facilitate the passing of highly selected pollen tubes across the interlocular compital space. In origin, structure and proposed function these thick-walled, intra-ovarian trichomes conform to characteristics typically assigned to obturators.

Twee-armige, eensellige epidermale trigome is karakteristiek van die Sapotaceae, maar is nog net gevind op uitwendige oppervlakke van bogrondse plantdele. In *Bequaertiodendron magalismontanum* (Sond.) Heine & J.H. Hemsl. kom soortgelyke trigome ook binne die stampers van volwasse vroulike en morfologies hermafroditiese blomme en in jong vrugte voor. Daar word voorgestel dat die interne trigome as brûe tussen die styl- en vrugbeginseltransmissieweefsel dien, om sodoende goedgeselekteerde stuifmeelbuise in staat te stel om die interlokulêre ruimte van die kompitum te oorbrug. In oorsprong, bou en voorgestelde funksie stem hierdie dikwandige, interne vrugbeginseltrigome ooreen met kenmerke wat vir obturators tiperend is.

Key words: *Bequaertiodendron magalismontanum*, compitum, intra-ovarian trichomes, ovarian transmitting tissue, pollen tube guides

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Introduction

The presence of bifurcate, unicellular epidermal trichomes is a very constant morphological feature of the Sapotaceae (Engler 1890; Metcalfe & Chalk 1950), an almost circumtropical family of woody perennials that bear edible and, usually, single-seeded berries. These thin- or thick-walled trichomes occur on the young shoots, leaves, pedicels and all floral whorls, except the petals. In many genera (Bhatnagar & Gupta 1970; Engler 1890; Roth & Lindorf 1972; Steyn & Robbertse 1990b) long-armed, thick-walled trichomes form a dense outer covering to the superior, syncarpous, multilocular ovaries of the insect-pollinated flowers. These trichomes may have evolved as additional protection against insect infestation that has been reported (Bradley 1981; Heppner 1981; Farooqi *et al.* 1973; Steyn & Robbertse 1990a) for several investigated taxa. During a detailed floral morphological/reproductive biological study, based on extensive field work in natural populations of *Bequaertiodendron magalismontanum* (Sond.) Heine & J.H. Hemsl., the only gynodioecious species (Steyn & Robbertse 1988, 1990a, 1990b, unpublished data) reported so far for the Sapotaceae, thick-walled, bifurcate trichomes were also found inside mature pistils and very young fruits. This extremely unusual characteristic has not yet been reported for *B. magalismontanum*. Neither have, to our knowledge, similar structures been mentioned previously for any other taxon in the literature. In this paper we investigate the location, origin and structure of the internal trichomes. In addition, we present an hypothesis, postulating that these structures form an important part of an intricate device for bringing about more successful fertilization of functional ovules in the multilocular ovary.

Material and methods

Freshly opened flowers from female plants with a high fruit:flower ratio (Steyn & Robbertse 1990a) during several consecutive seasons and from morphologically hermaphroditic, but functionally male plants (fruit:flower ratio less than 56:1000 during the same period), as well as post-anthesis stages of these flowers, were fixed and stored in a long-term fixative for light microscopical studies (Collins & MacNichol 1978). In order to improve the fixation of tannin-containing tissues, 0.5% caffeine was added, following the procedures of Mueller & Greenwood (1977), to the phosphate-buffered, 5% paraformaldehyde solution. Conventional methods were applied for obtaining serial, GMA (glycolmethacrylate) sections (*ca.* 2 μm) of ovaries and young fruits for light microscopy. Selected GMA sections, after staining with PAS/toluidine blue, were treated with aniline blue and examined under fluorescent light for the possible presence of pollen tubes (Smith & McCully 1978). For SEM studies, ten female and ten functionally male plants were selected randomly and 20 open flowers from each plant were dissected to determine the relative sex ratio for ovaries containing trichomes. Trichome-bearing ovules, carefully removed from the ovaries, were prepared for SEM studies according to conventional procedures.

Observations

To understand the operation of the intra-ovarian trichomes and their role in the reproductive process, it is necessary to

consider some characteristics of the components comprising the device that possibly ensures fertilization of functional ovules in the multilocular ovaries of *B. magalismontanum*.

Position of the compitum

The wet stigma is divided into as many lobes as there are locules in the ovary (five in hermaphroditic, four to ten in female flowers). Each papillate stigmatic surface leads into its own stylar canal that is lined by a layer of small, thin-walled, densely cytoplasmic, papillate cells. These cells therefore form separate tracts that constitute the stylar transmitting tissue (Figure 1) within the open style (Knox *et al.* 1986). In the transition region between the lanceoloid style and the ovoid ovary the separate tracts converge to form a single, compound tract that appears stellate in transverse sections (Figure 2) with 'arms' lined by transmitting tissue. Lower down, the stellate transmitting tract leads into an intragynoecial, interlocular space in the distal part of the ovary (Figures 3 and 5). This space is the result of the ventral margins of the carpels being unfused in this region and not continuous with the central axis of the ovary (Figures 3, 4 and 5), so that the septa between the ovules are incomplete. The locules with their single, tight-fitting ovules are therefore interconnected through narrow channels, each representing one arm of the stellate transmitting tract, that lead into an interlocular space (Figures 3, 4 and 5). The chalazal parts of the ovules have stomata in the dorsal epidermis (Steyn & Robbertse, unpublished data), suggesting that the ovarian cavity and the interlocular space contain air. In the transition region between the style and ovary a paracarpous region or compitum (Carr & Carr 1961) therefore occurs, comprising a compound pollen tube transmitting tract and an intragynoecial, interlocular air-space.

Placentation and structure of ovules

The anatropous, unitegmic, sessile ovules are attached to the central axis of the ovary for a considerable distance along their ventral parts (Figures 6 and 7), as reported for several advanced taxa of the Sapotaceae (Corner 1976). Each ovule is curved inwards, i.e. towards its ventral side, so that the rimiform, vertically orientated, ventrally displaced micropyle lies close to the lower (micropylar) junction of the ovule to the central axis (Figure 7). The micropyle lies in the same vertical plane as the corresponding radiating arm of the stellate transmitting tract higher up, but is separated from the stylar transmitting tissue by the interlocular space and the broad placental region. Unstained, mature ovules from female and hermaphroditic flowers have a narrow, colourless epidermal region encircling the placenta, i.e. the region of attachment to the central axis. At the micropylar side this ring of tissue broadens and stretches between the lower junction of the ovule to the placenta and the micropyle. Serial sections of mature ovules show that ovular epidermal cells adjacent to the lower junction of the ovule to the placenta are thin-walled, slightly papillate, contain less tannin than adjacent epidermal cells and, in ovules with normally developed embryo sacs, secrete droplets of an unidentified substance (Figures 6 and 8). These secreting cells closely resemble the cells of the stylar transmitting tissue. We suggest that they are part of the colourless epidermal ring encircling the placenta and represent ovarian transmitting tissue (Tilton & Horner 1980) that provides nutritive and possibly chemotropic materials for pollen tubes (Figure 8) during their growth towards the micropyle.

Location, structure and origin of intra-ovarian trichomes

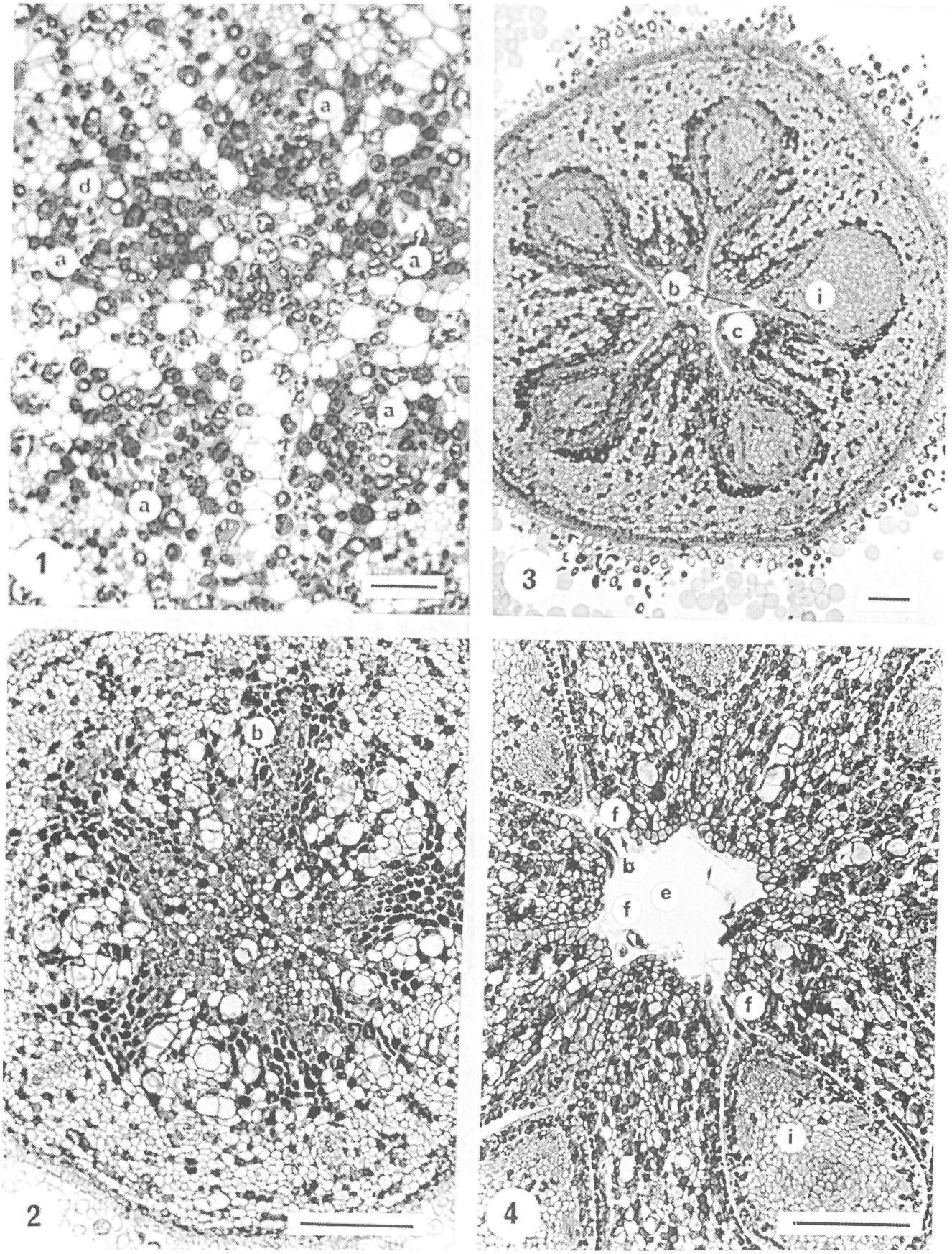
The thick-walled trichomes of *B. magalismontanum* occur inside the compitum (Figure 10) and resemble mature, extra-ovarian trichomes (Figures 7, 13, 14 and 15). The thickened, unlignified outer wall encloses a tannin-containing lumen. Nuclei are absent. The arms that taper towards the ends are approximately of equal length (100 – 250 µm.) The short base (Figures 5, 10 and 12) is implanted into a very localized region of the ovular epidermis, namely at the chalazal junction of the sessile ovule to the placenta of the central axis (Figures 5 and 12). One arm usually extends upwards (Figures 5 and 12) into the stellate tract, or for some distance stretches along horizontally, close to the chalazal ovular epidermis (Figures 9, 11 and 12), while the other leads down towards the micropyle (Figures 11 and 13).

Only one trichome develops per ovule, but not every ovule has a trichome. Trichomes were more often found inside ovaries from female flowers (85%) than inside ovaries from hermaphroditic flowers (32%). In the latter, trichomes often have thinner cell walls (Figure 5) and occurred on fewer ovules per ovary, but a relative sex ratio for trichome-bearing ovules could not be obtained, as removal of tight-fitting ovules from ovaries could have caused breaking of delicate trichomes. In serial sections of ovules with degenerated embryo sacs trichomes were not found and they were sometimes also absent on ovules with fully developed embryo sacs. Ovules that had been fertilized, judged by the presence of pollen tubes in the vicinity of the micropyle (Figure 8), have trichomes. In young, single-seeded fruits from female plants a trichome was always present on the developing seed, but sometimes also occurred on one or more of the ovulodes (Figures 14 and 15).

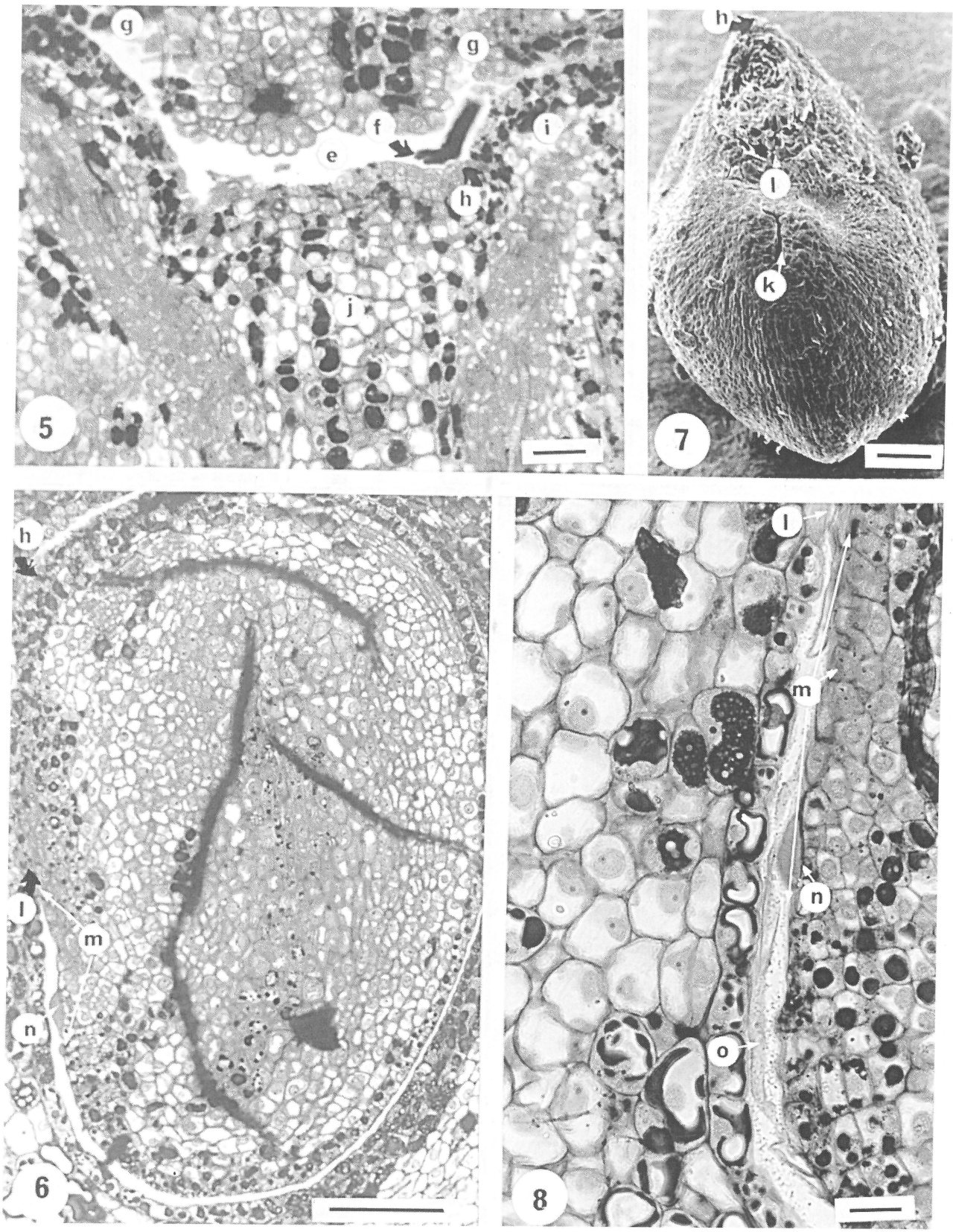
Discussion

In contemporary insect-pollinated plants with eusyncarpous gynoecia (i.e. syncarpous gynoecia with a paracarpous region or compitum) like *B. magalismontanum*, the transmitting tissue of the stigma and style serves as a unique selective mechanism for male genomes with a high degree of metabolic vigour (Mulcahy 1979; Endress 1982; Endress *et al.* 1983). Large amounts of pollen, deposited simultaneously on stigmatic surfaces by pollen vectors, induce competition among non-random microgametophytes (Galen *et al.* 1986 and references therein; Mulcahy 1979). The selective pressure exerted upon the pollen tubes is intensified during their growth through stylar canals and less fit male gametophytes are screened out (Mulcahy 1979). In the compound transmitting tract competition for male genomes with the most vigorous properties is further reinforced (Endress 1982). The lower the position of the compitum, the higher the selection pressure (Ramp 1987). The paracarpous nature of the compitum enables pollen tubes from any stigma or part of a compound stigma to cross over and fertilize any ovule in any locule of a multicarpellary ovary (Carr & Carr 1961).

An important function of transmitting tissue is to provide nutrients and, possibly, chemotropic substances to pollen



Figures 1 – 3 Transverse sections of pistil of hermaphroditic flower. 1. Apical part of style. 2. Transition region between style and ovary. 3. Distil part of 5-locular ovary. a, separate stylar canals; b, stellate transmitting tract; c, free ventral carpel margin; d, papillate transmitting tissue; i, ovule. All scale bars: 100 μm. **Figure 4** Transverse section of ovary of female flower at slightly lower level than Figure 3. b, stellate transmitting tract; e, interlocular space; f, intra-ovarian trichome; i, ovule. Scale bar: 100 μm.



Figures 5 – 8 5. Longitudinal section of ovary of hermaphroditic flower illustrating origin of trichome and opening of stellate tract leading into interlocular space of compitum. 6. Off-median longitudinal section of same ovule (i) shown in Figure 5. 7. Scanning electron micrograph of dissected ovule in ventral view, illustrating curvature towards ventral side and position of micropyle. 8. Close-up view of ovarian transmitting tissue and adjacent cell layers of same ovule (i) shown in Figures 5 and 6. e, interlocular space; f, intra-ovarian trichome; g, opening of stellate tract; h, upper junction of ovary to central axis; i, ovule; j, central axis; k, micropyle; l, lower junction of ovule to central axis; m, ovarian transmitting tissue; n, secretion droplet; o, pollen tube. Scale bars: 50 μm in Figures 5 – 7 and 10 μm in Figure 8.

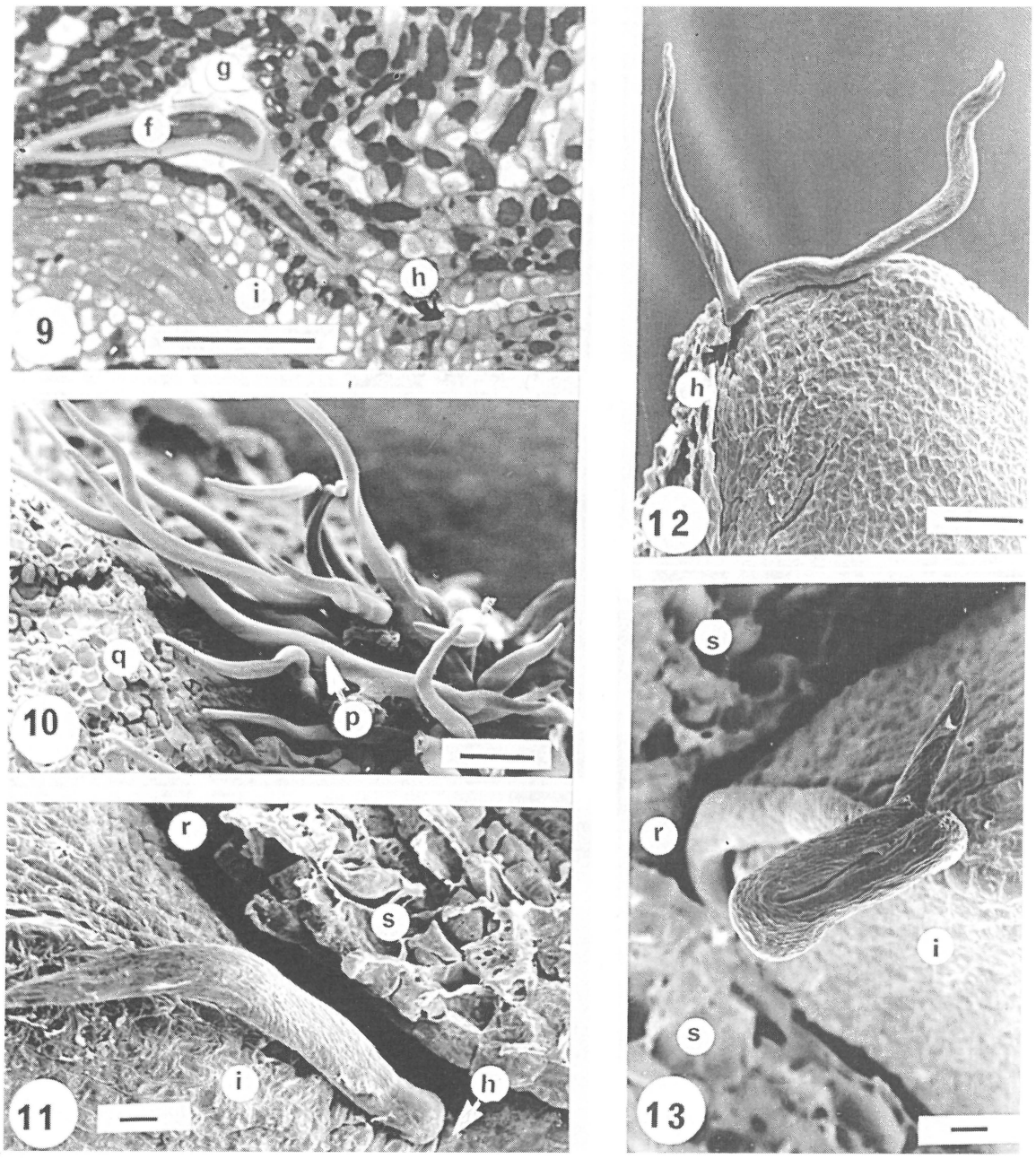
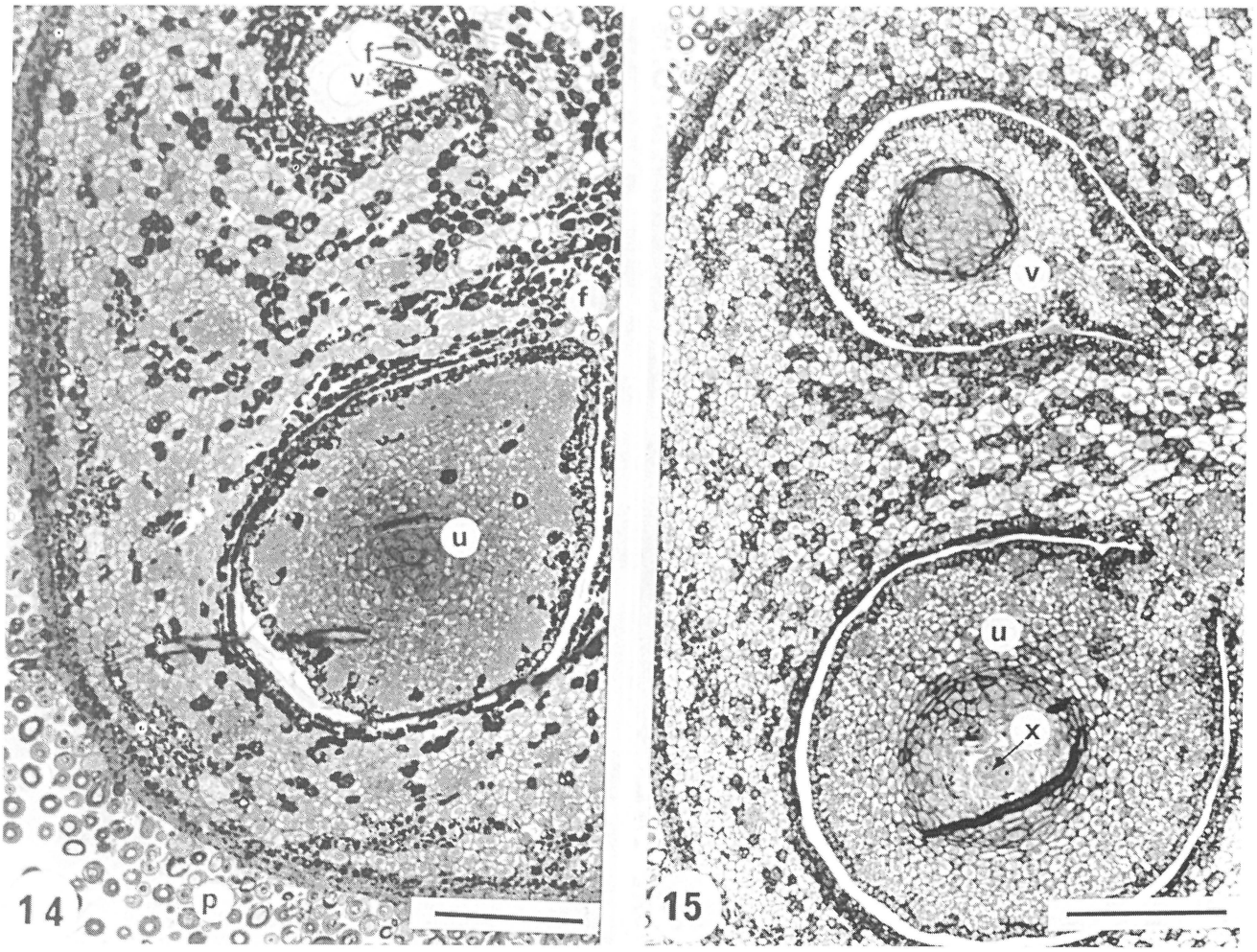


Figure 9 Longitudinal section of upper part of ovule of female flower, illustrating thick-walled trichome in opening of stellate tract — compare with Figure 5. f, intra-ovarian trichome; g, opening of stellate tract; h, upper junction of ovule to central axis; i, ovule. Scale bar: 50 μm. **Figures 10 – 13** Scanning electron micrographs of bifurcate trichomes. 10. Part of ovary wall with extra-ovarian trichomes. 11 – 13. Upper parts of ovules with intra-ovarian trichomes. 11. Trichome with one arm orientated horizontally, the other arm (not visible) growing down towards micropyle. 12. Trichome with arms extending upwards. 13. Trichome with one arm growing upwards, the other disappearing towards micropyle. h, upper junction of ovule to central axis; i, ovule; p, extra-ovarian trichome; q, ovarian wall; r, ovarian cavity; s, septum between ovules. Scale bars: 50 μm in Figures 10 and 12 and 10 μm in Figures 11 and 13.

tubes during their growth to the micropyle (Carr & Carr 1961; Dalmer 1880; Tilton & Horner 1980). The cuticle covering these cells is either extremely thin, or lifted up by secretions from the underlying cells. The greater availability of nutrients and possible chemotropic substances from these cells than from neighbouring tissues may explain the lack of growth of pollen tubes over the internal, cutinized surface of the ovary and its associated structures (Carr & Carr 1961; Dalmer 1880).

Forces guiding the growth of pollen tubes along the transmitting tissue are not well understood. Thigmotropism certainly plays a role, as pollen tubes tend to follow cell surfaces or inert objects and may be guided through the ovary by furrows, trichomes or guide structures such as obturators (Knox *et al.* 1986). Chemotropism has been suggested by several authors (Mascarenhas & Machlis 1962, 1964; Mulcahy & Mulcahy 1985, 1987; Peterson *et al.* 1979; Tilton & Horner 1980; Robbertse *et al.* 1990). Substances responsible for this tropism are possibly minerals like calcium and boron, or an interaction between these two substances, that stimulate and direct pollen tube growth towards the ovary (Robbertse *et al.* 1990 and references



Figures 14 and 15 Transverse sections of young fruits from female plants. 14. Distal part of fruit. 15. Central part of fruit shown in Figure 14, illustrating same developing seed and adjacent ovulode. f, intra-ovarian trichome; p, extra-ovarian trichome; v, ovulode; x, embryo. Scale bars: 100 μm.

therein). The ovary probably acts as a boron sink during flower maturation (Robbertse *et al.* 1990). Robbertse *et al.* (1990) also demonstrated that pollen tubes consistently follow the shortest route towards the boron supply.

We suggest that, because of the selective mechanism operating in the stigma and style and the low position of the compitum, very few pollen tubes enter the interlocular air-space in the distal part of the ovary of *B. magalismontanum*. The actual number could not be determined in the thin serial sections necessary for anatomical details, probably because the fluorescent reaction of the thin pollen tubes is too weak and of short duration. Because of the paracarpous nature of the compitum, the selected pollen tube can cross over to any locule of the multicarpellary ovary that contains an ovule with a functional embryo sac. If pollen tubes are thigmotropic, the selected pollen tube probably would enter the narrow ovarian cavity and cross over onto the surfaces of ovules that fit tightly into the locules, but these epidermal, cutinized surfaces would not sustain their growth. Because the stylar transmitting tissue is separated from the ovarian transmitting tissue by the interlocular space, the pollen tube cannot reach the last-mentioned nutritive tissue that would facilitate growth towards the micropyle of a functional ovule. Fertilization of a functional ovule therefore requires the presence of a bridgelike structure or ponticulus (Joel & Eisenstein 1980; de Wet *et al.* 1990) ensuring passage of pollen tubes towards the micropyle.

Conclusions

We propose that the thick-walled trichomes serve as bridges between stylar and ovarian tissues in the pistil of *B. magalismontanum* to facilitate the passing of pollen tubes across the interlocular air-space and to guide the tubes along the shortest route towards the micropyle of a functional ovule. Chemotropic and thigmotropic forces are probably both involved in the crossing process, as pollen tubes tend to follow guide structures (thigmotropism) and stylar and ovarian transmitting tissues, to which the arms of the trichomes closely adhere, possibly providing chemotropic substances. Indicators in accordance with our hypothesis are: the higher incidence of trichome-containing ovules in female plants with a high fruit:flower ratio than in functionally male plants; the presence of trichomes on ovules with functional embryo sacs in hermaphroditic and female plants; the absence of trichomes on ovules with degenerated embryo sacs in hermaphroditic plants and the presence of trichomes on developing seeds in young fruits.

In recent forms of the species the characteristics of intra-ovarian trichomes correspond to those typically assigned to obturators (Bouman 1984; Tilton & Horner 1980). These bi-

furcate, individually occurring trichomes of *B. magalismontanum* may represent ancestral, multitrichomal obturatory structures that have been reduced to the bare essentials in gynodioecious populations of an originally tropical and hermaphroditic species, now struggling for survival in the hostile environment at the southern extreme of the species' range.

It was proposed previously (Steyn & Robbertse 1988) that embryological data on seemingly hermaphroditic plants may provide morphological evidence of female sterility in these plants. Such evidence was found in the present investigation. Although the ovaries of morphologically hermaphroditic plants contain five well-developed ovules, in several of the latter the embryo sacs are non-functional. This may indicate that a second modifier (Charlesworth & Charlesworth 1978) or female sterility factor has already been introduced into gynodioecious populations of this species, turning some individuals into males. The reasons for the positive correlation between functional ovaries and the presence of intra-ovarian trichomes are not clear, but it may be possible that the second modifier has a detrimental effect on the development of embryo sacs as well as intra-ovarian trichomes. This aspect warrants further investigation.

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